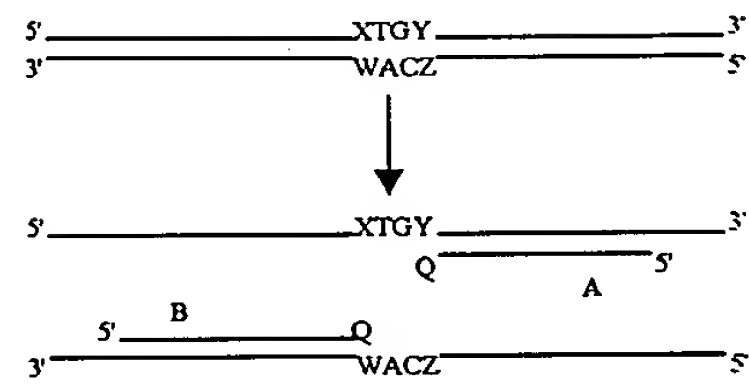
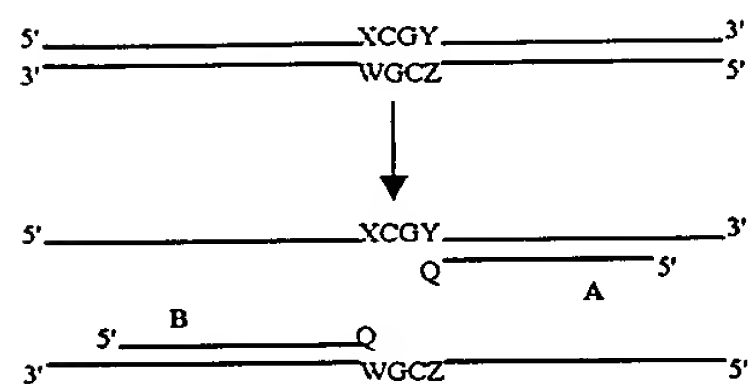


# STEPS IN PCR/RE/LDR

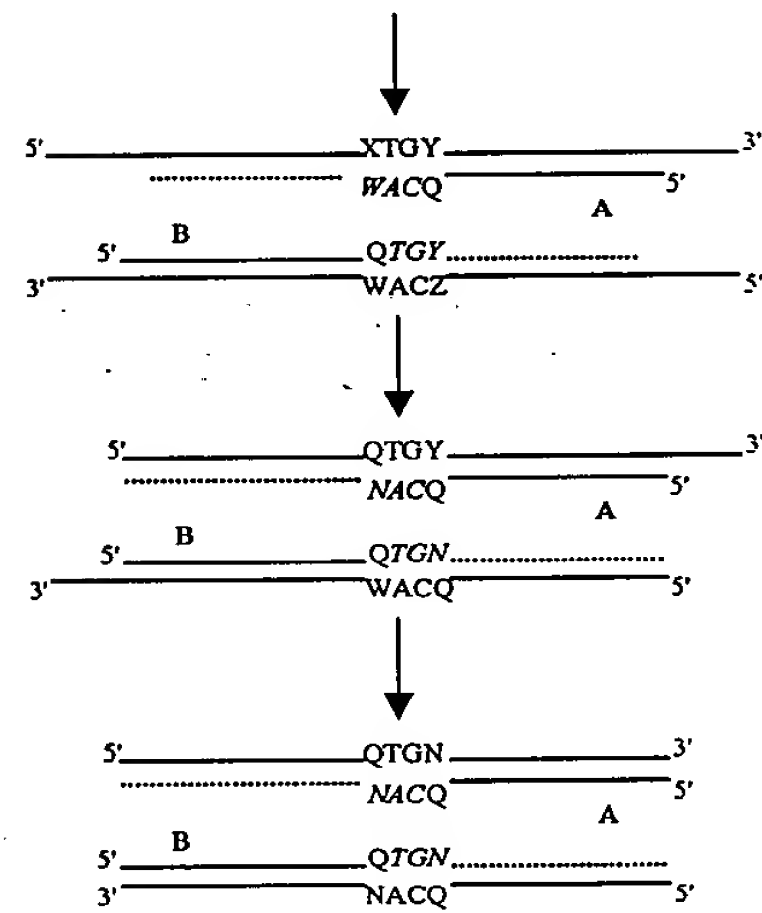
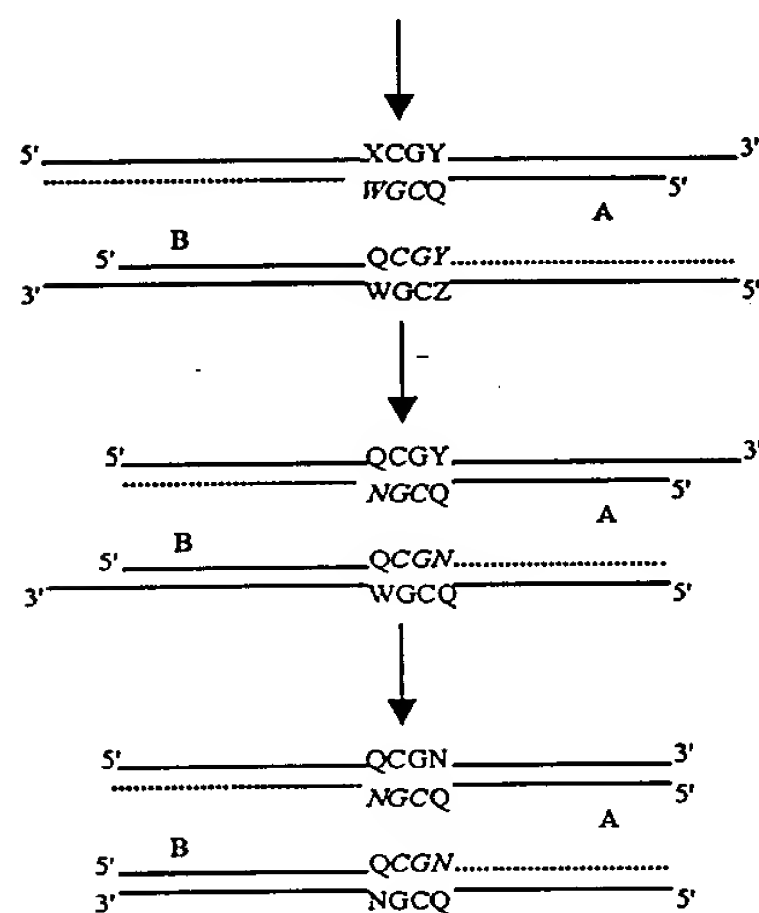
## WILD-TYPE

## MUTANT

1)



2)



3)

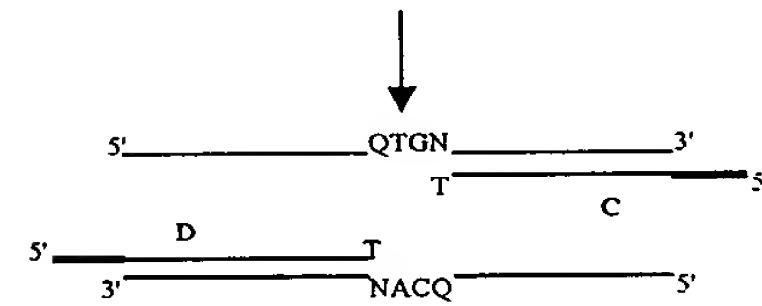
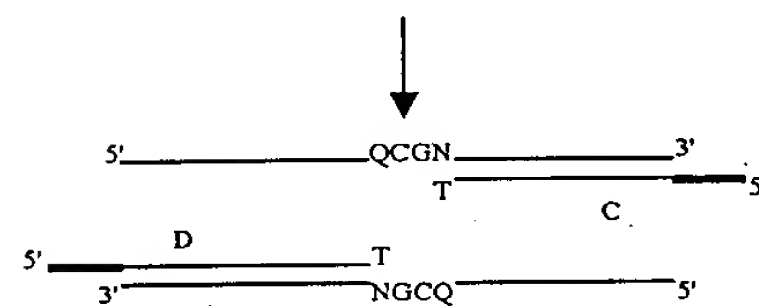
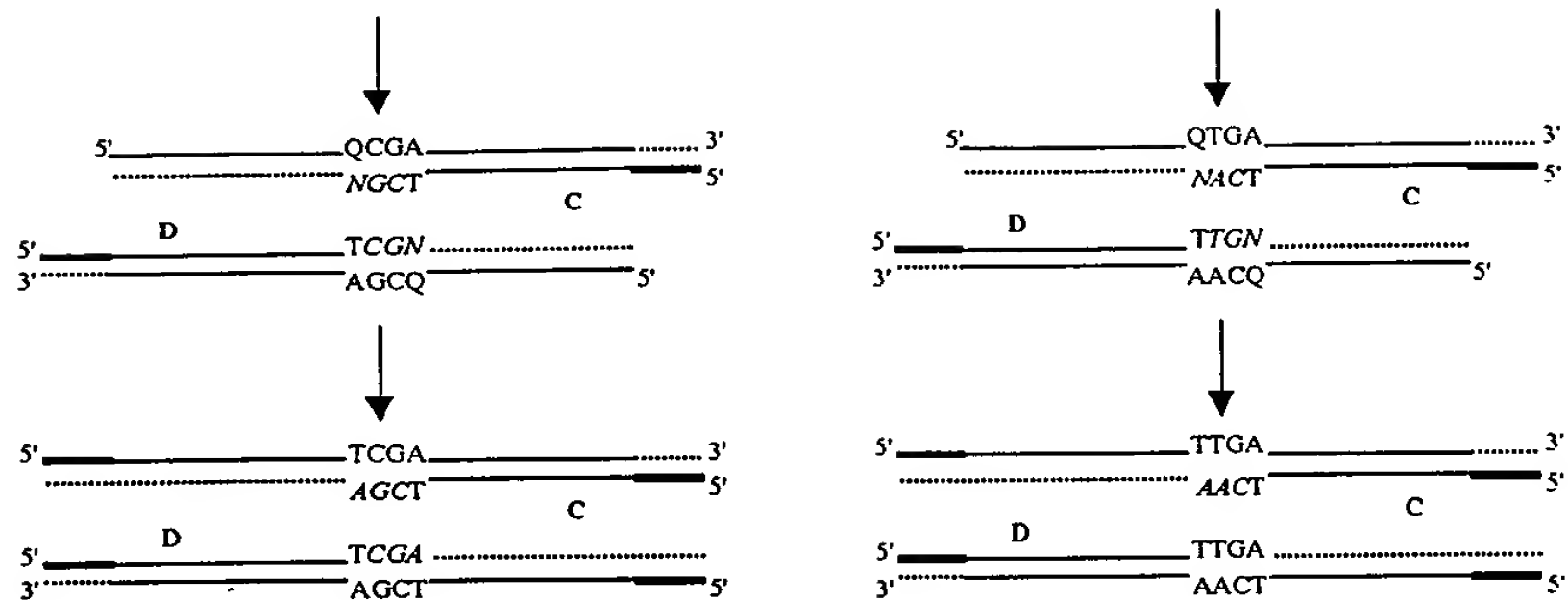


FIGURE 1

4)



5)

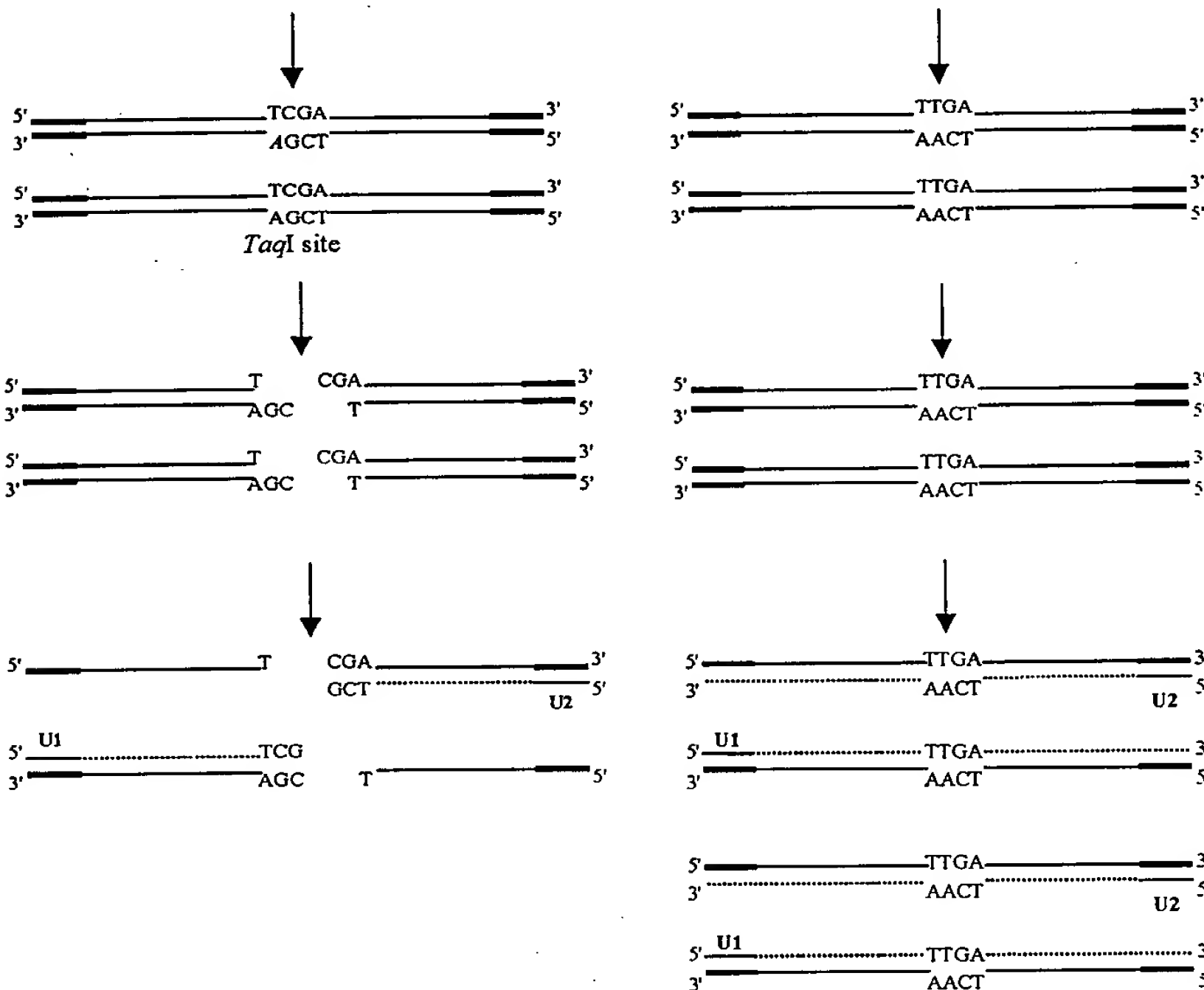
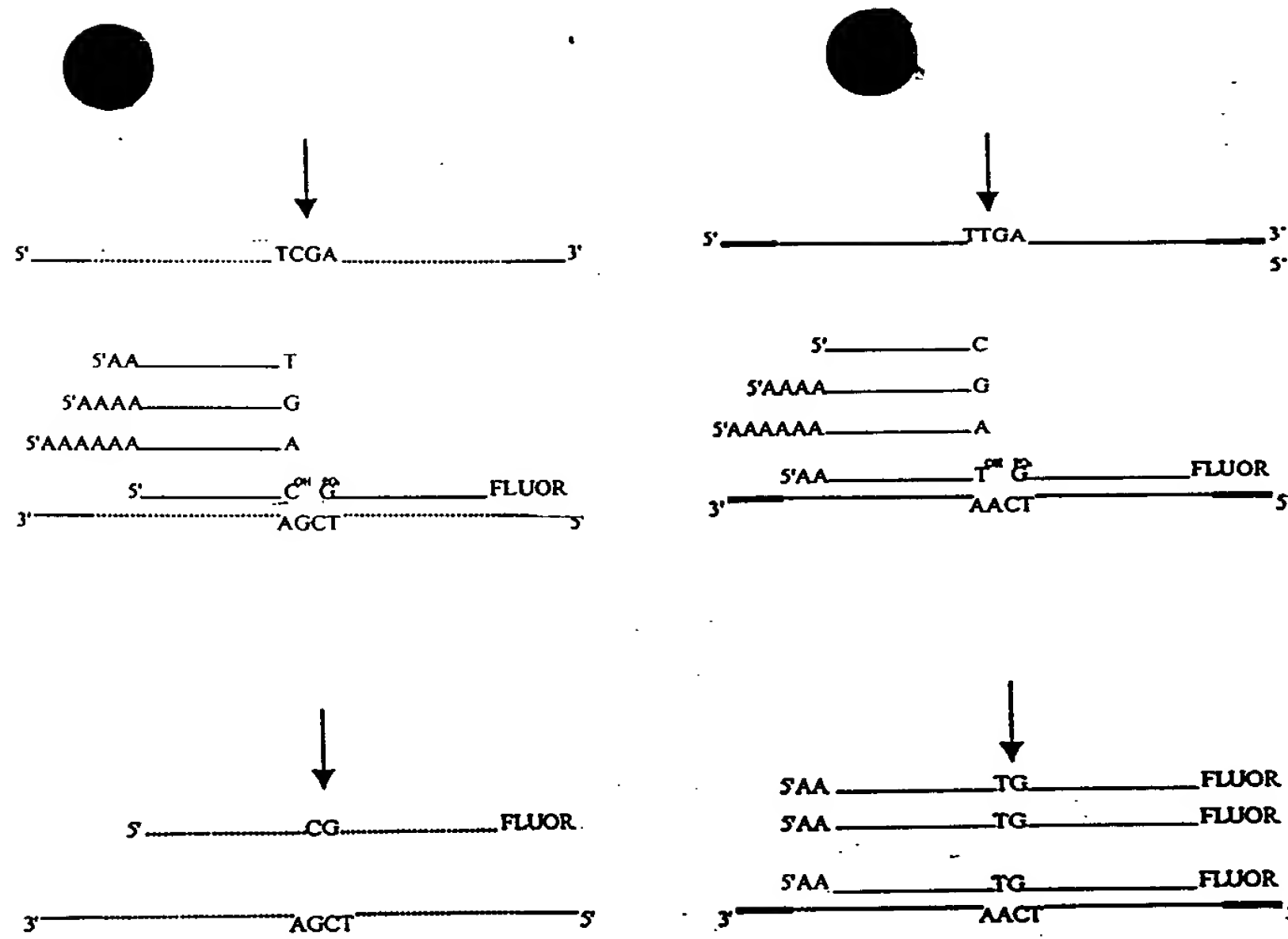


FIGURE 1 (Cont'd.)

7A)



From 6)

From 6)

7B)

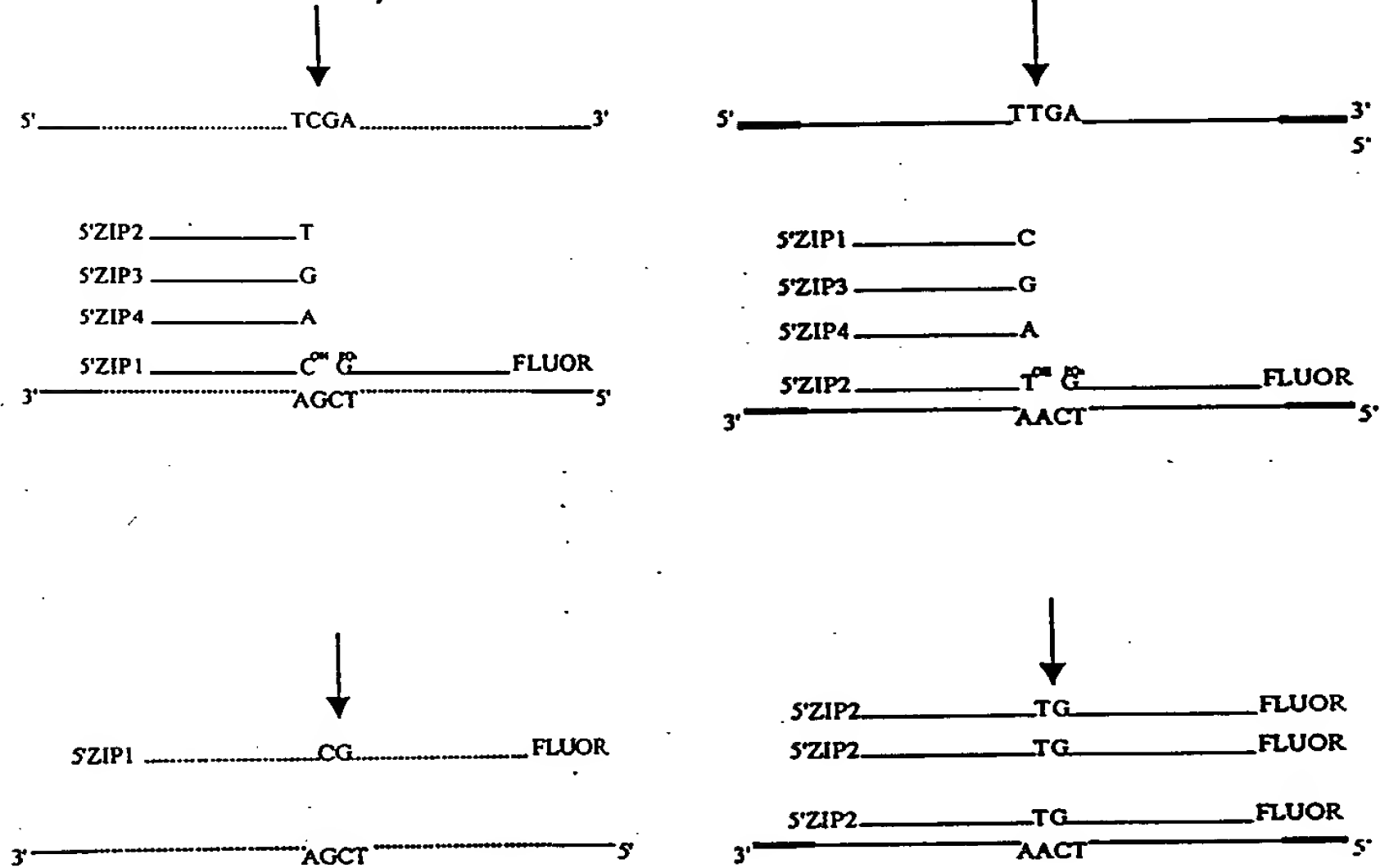


FIGURE 1 (Cont'd.)

002 FEB 74 11 23 AM

SAA ——— TG ——— FL

S ..... CG ..... FL

S'AAAAAA ——— A

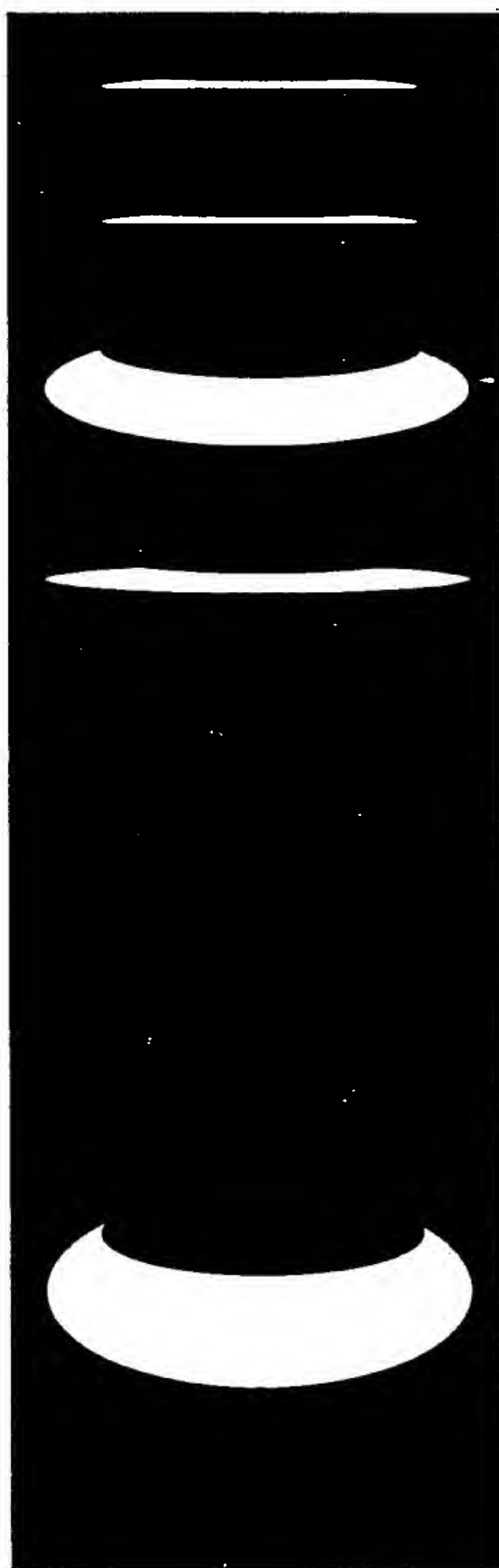
S'AAAA ——— G

S'G ..... FL

S'AA ——— T

S' ——— C

I. GEL LANE



INCREASING MASS

FIGURE 2

5'ZIP2 \_\_\_\_\_ TG \_\_\_\_\_ FLUOR


5'ZIP1 ..... CG ..... FLUOR

5'ZIP2 \_\_\_\_\_ T

5'ZIP3 \_\_\_\_\_ G

5'ZIP4 \_\_\_\_\_ A      G \_\_\_\_\_ FLUOR

5'ZIP1 \_\_\_\_\_ C

 Apply sample to Array

G\_\_\_\_\_FLUOR

## Wash

G\_\_\_\_\_FLUOR

Dark

Read Array fluorescence

(3)

## Hybridize Array

(2)

FIGURE 3

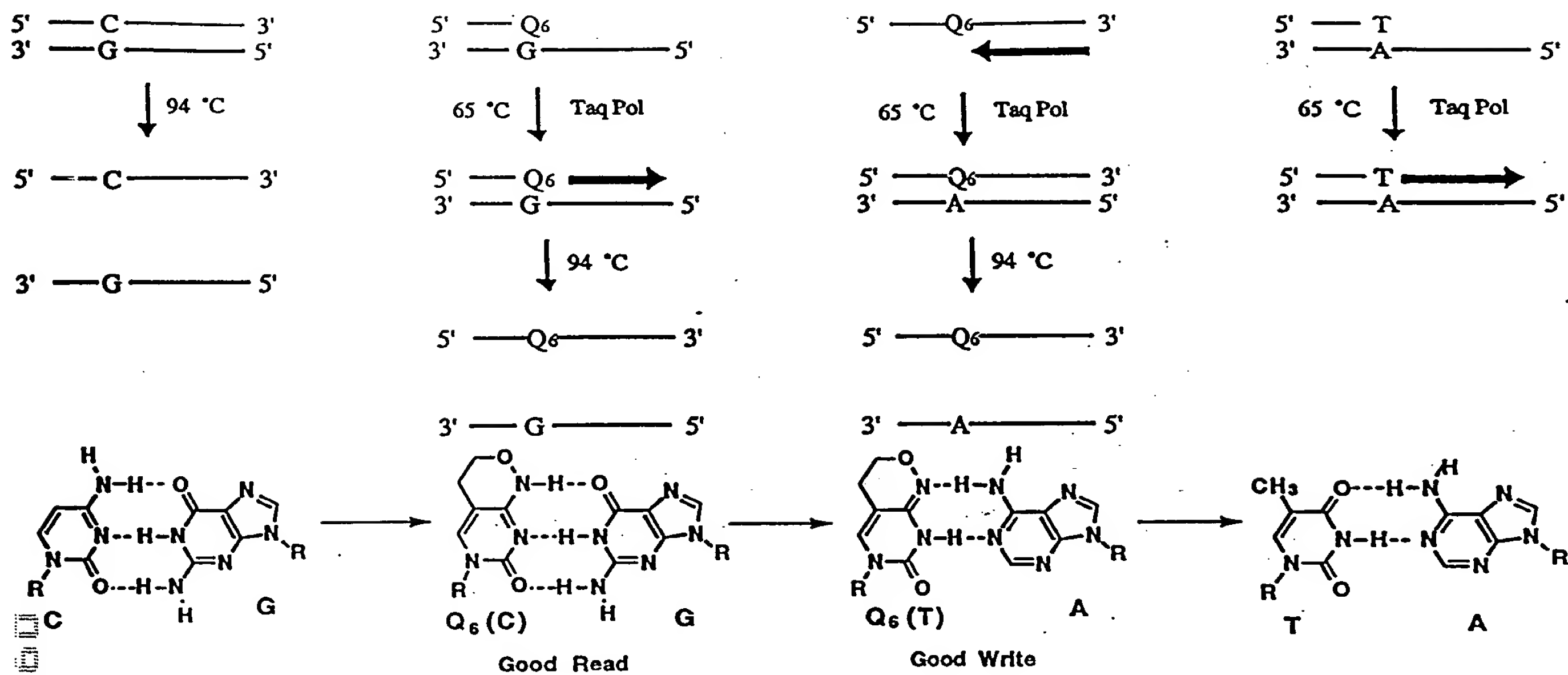


FIGURE 4

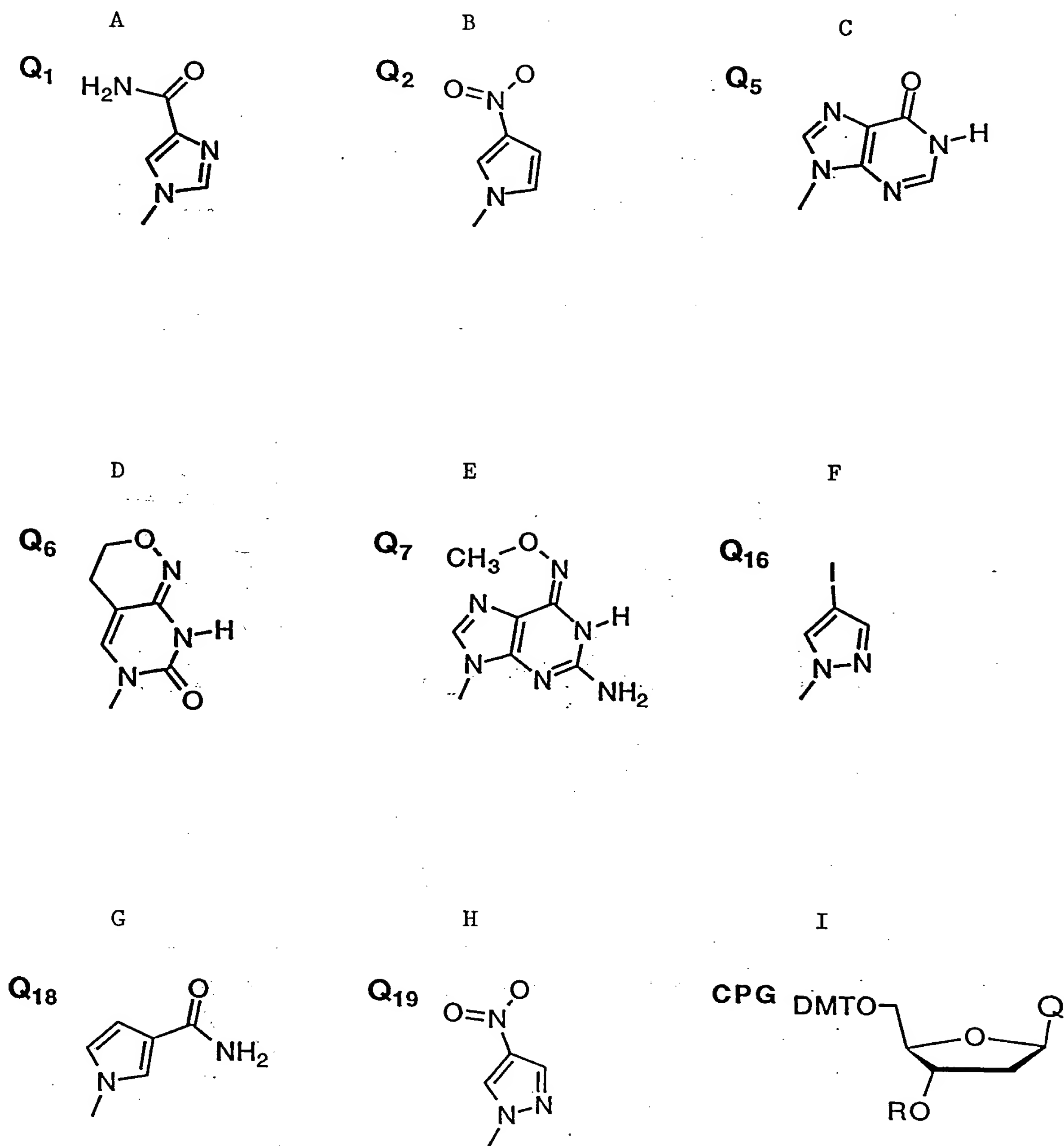
[illegible]

FIGURE 5

**A**

**Primers**

**Ztop**

CTT GGA OGA GTT CAT ACG C

codon 248



**p53zip248**

CTT GGA OGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A

**p53-248X**

T TCT TCC TGC ATG GGC GGC ATG AAX→*pol*

**50 bp synthetic duplex DNA**

|| ||| ||| ||| ||| ||| ||| |||

3' CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC GGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| ||| |||

*pol*-x TCC GGG TAG GAG TGG TAG TAG TCT T

**p53-248XR**

**p53zip248R**

C GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC G

**Zbot**

C ACC GCT GGG TCA AAC G

**B**

**Primers**

**Ztop**

CTT GGA OGA GTT CAT ACG C

codon 248



**p53zip248T**

CTT GGA OGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG AAT

**p53-248Q<sub>N</sub>**

T TCT TCC TGC ATG GGC GGC ATG AAQ<sub>N</sub>→*pol*

**50 bp synthetic duplex DNA**

|| ||| ||| ||| ||| ||| ||| |||

3' CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC GGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| ||| |||

*pol*-Q<sub>N</sub> TCC GGG TAG GAG TGG TAG TAG TCT T

**p53-248Q<sub>N</sub>R**

**p53zip248TR**

T TCC GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC G

**Zbot**

C ACC GCT GGG TCA AAC G

**C**

**LDR Primers**

**p53LDR248FCA**

F-AAAAAA GC ATG GGC GGC ATG AAC A

**p53LDR248FCG**

F-AAAA GC ATG GGC GGC ATG AAC G

**p53LDR248FCT**

F-AA GC ATG GGC GGC ATG AAC T

**p53LDR248FCC**

F- GC ATG GGC GGC ATG AAC C *7-ligase*

**p53LDR248PGG**

GG AGG CCC ATC CTC ACC ATC AT-block

**conversion products**

3' (- strand)

... GTA TGC GCA AGG ACG TAC CCG CCG TAC TTG ACC TCC GGG TAG GAG TGG TAG TAG TGA ACC...

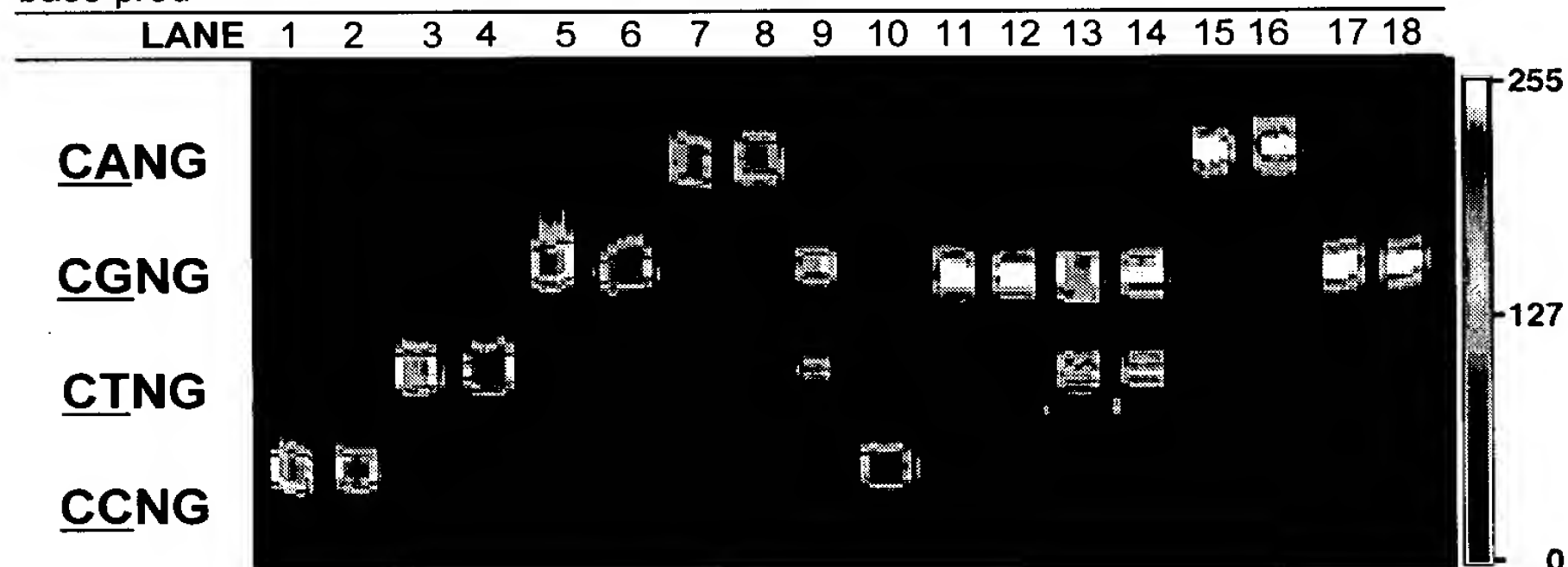
5'

FIGURE 6



**A**

Template	CCGG		CTGG		CGGG		CAGG		TCGA		GCGC		ACGT		CATG		CGCG	
Expctd prod	<u>CCGG</u>		<u>CTGG</u>		<u>CGGG</u>		<u>CAGG</u>		<u>CCGG</u>		<u>CCGG</u>		<u>CCGG</u>		<u>CATG</u>		<u>CGCG</u>	
primer 3' end	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>	C	Q <sub>6</sub>
1st base	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2nd base	C	C	T	T	G	G	A	A	G	C	G	G	G	G	A	A	G	G
minor 2nd									t		t		t		T		T	
base prod													c		c		c	



**B**

Template	CCGG		CTGG		CGGG		CAGG		TCGA		GCGC		ACGT		CATG		CGCG	
Expctd prod	<u>TCGA</u>		<u>TTGA</u>		<u>TGGA</u>		<u>TAGA</u>		<u>TCGA</u>		<u>TCGA</u>		<u>TCGA</u>		<u>TATA</u>		<u>TGCA</u>	
primer 3' end	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>	T	Q <sub>6</sub>
1st base	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
2nd base	?	C	?	T	?	G	?	A	C	C	c	C	?	C	?	A	?	G
minor 2nd											?	?	c	?	g		g	
base prod																		

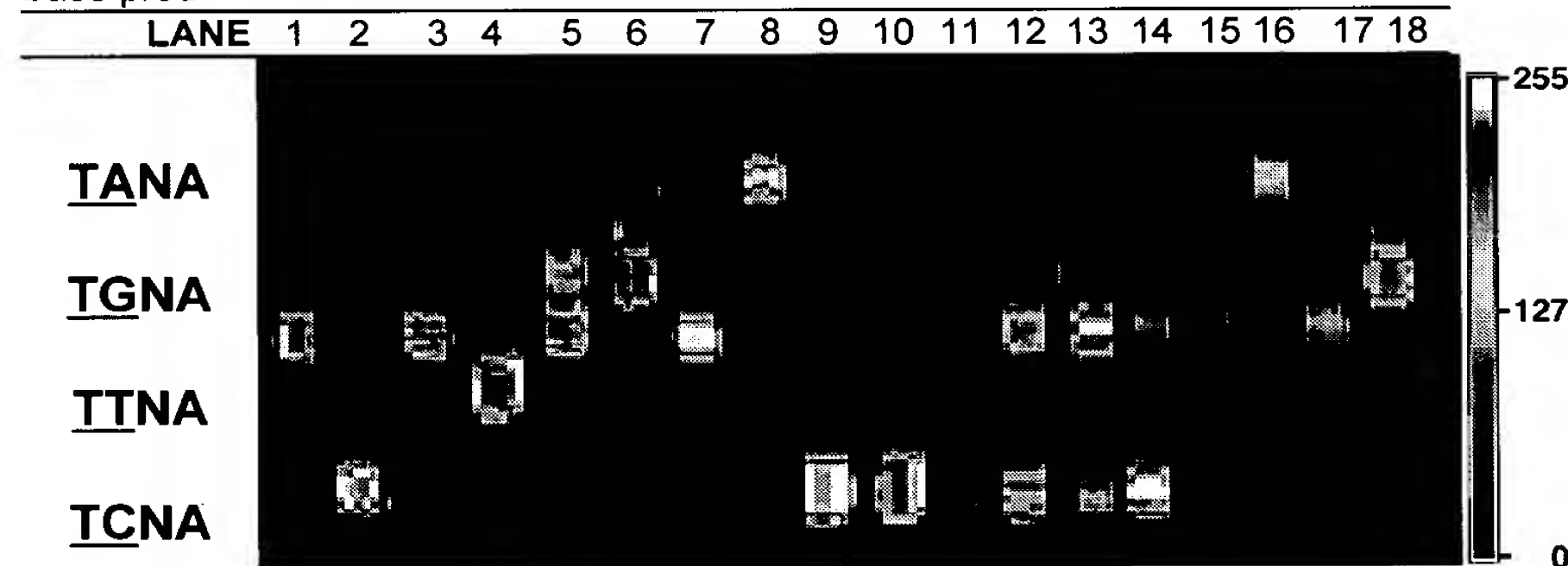
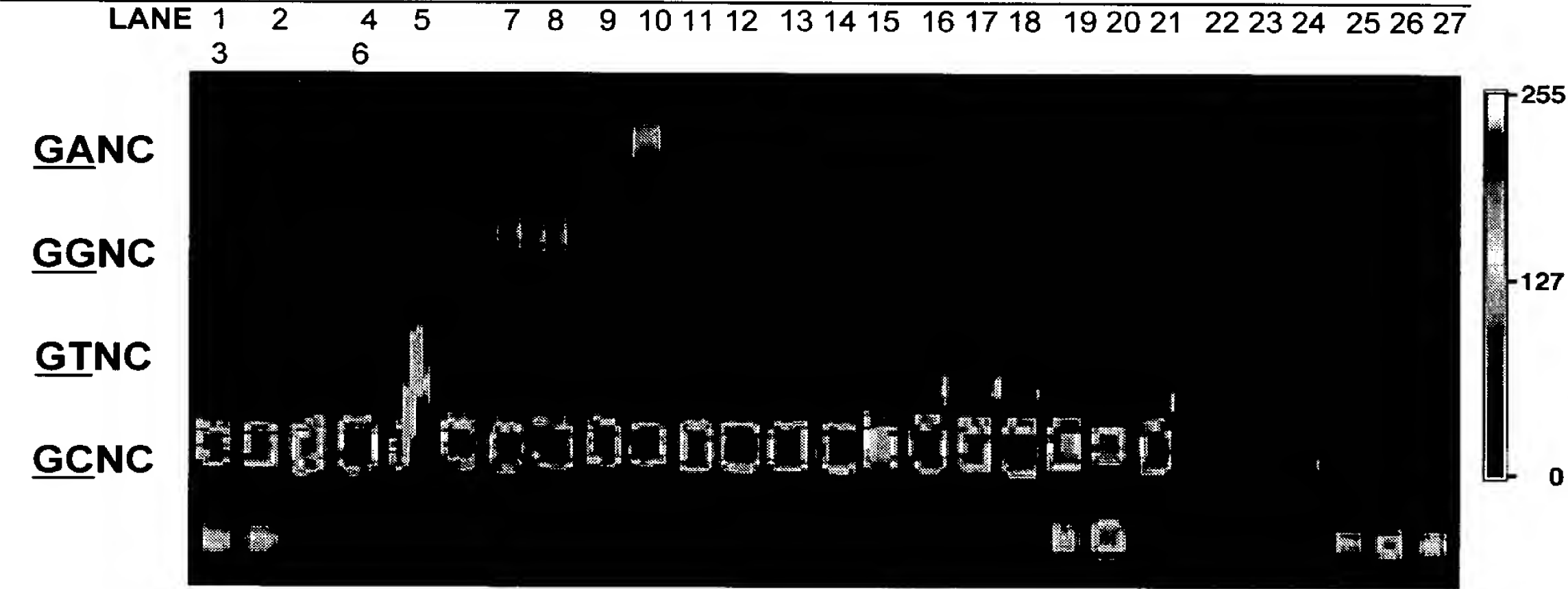


FIGURE 7

A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	GCGC	GTGC	GGGC	GAGC	GCGC	GCGC	GCGC	GATC	GGCC
primer 3' end	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>	G Q <sub>5</sub> Q <sub>7</sub>
1st base	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	? ? ?
minor 2nd base prod		t t t	g g	A A a					



B

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	ACGT	ATGT	AGGT	AAGT	ACGT	ACGT	ACGT	AATT	AGCT
primer 3' end	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>	A Q <sub>7</sub> Q <sub>5</sub>
1st base	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	c C c
minor 2nd base prod	?		?						

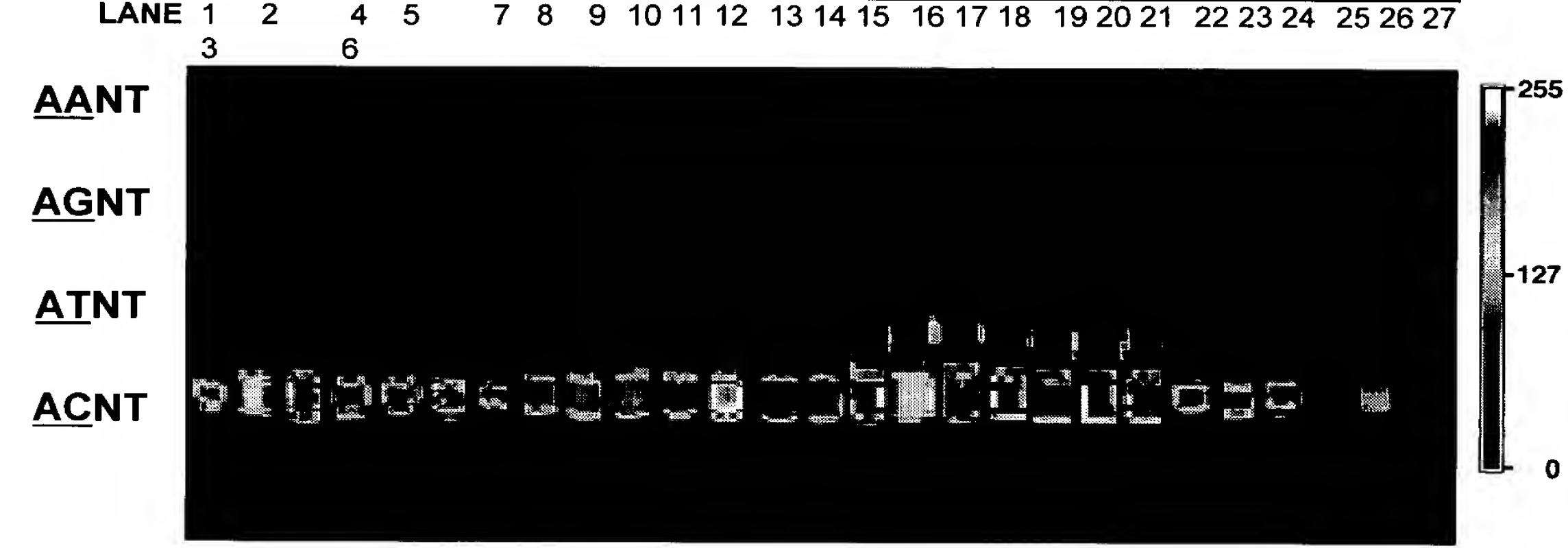
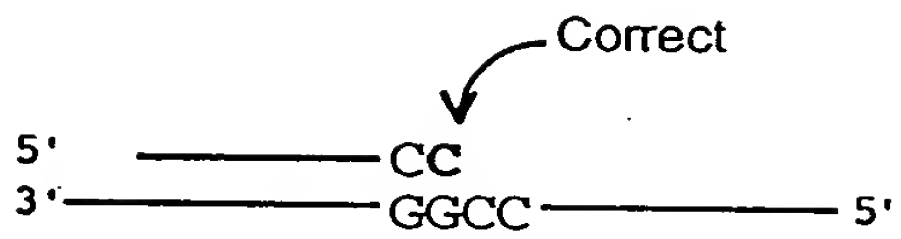
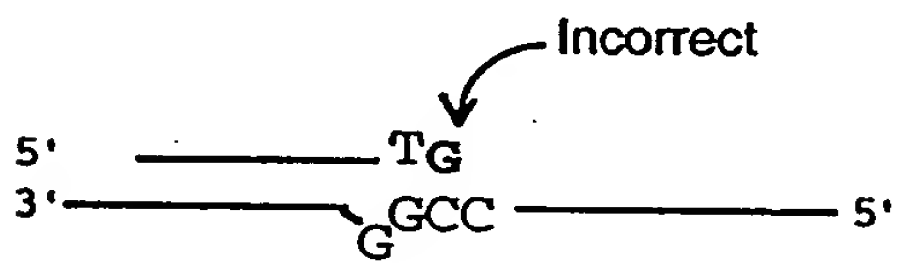


FIGURE 8

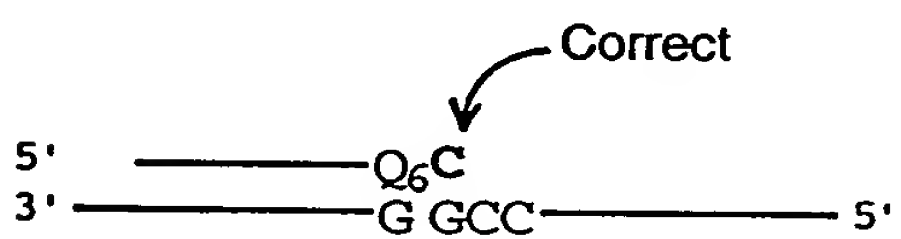
A



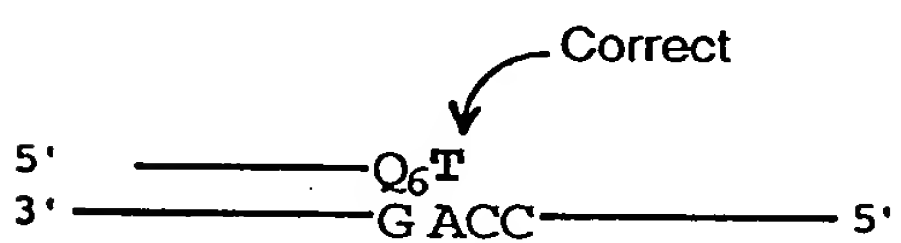
B



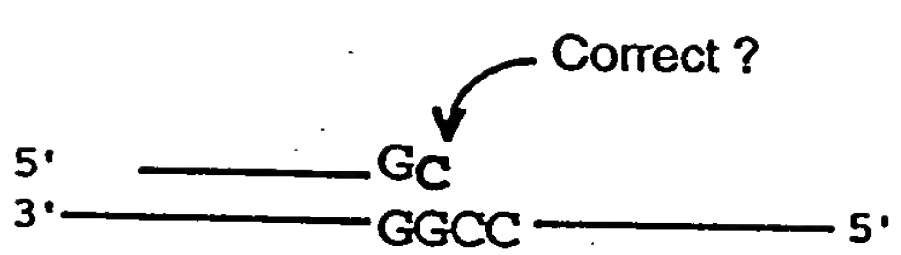
C



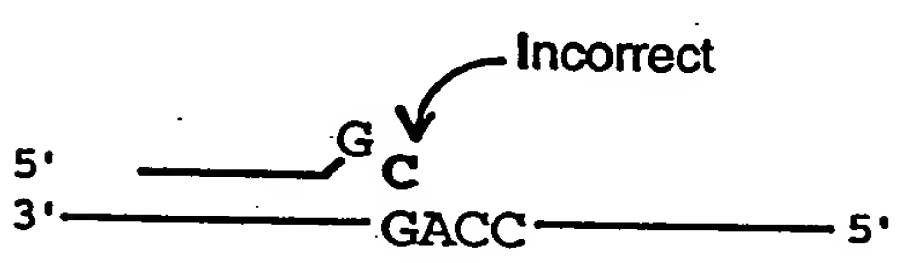
D



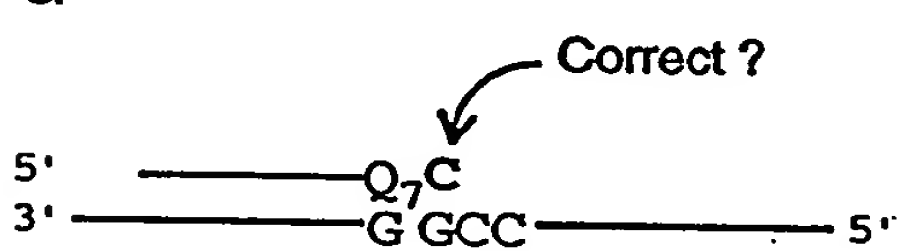
E



F



G



H

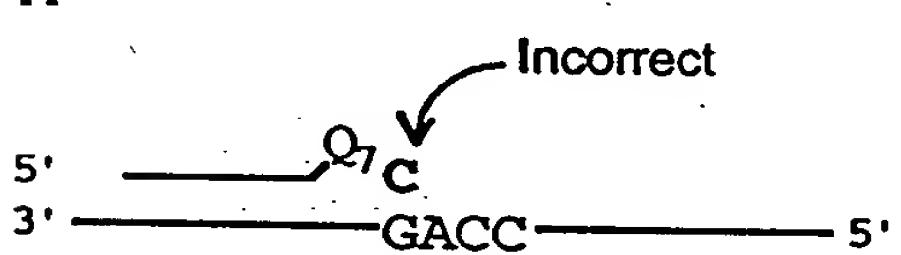


FIGURE 9

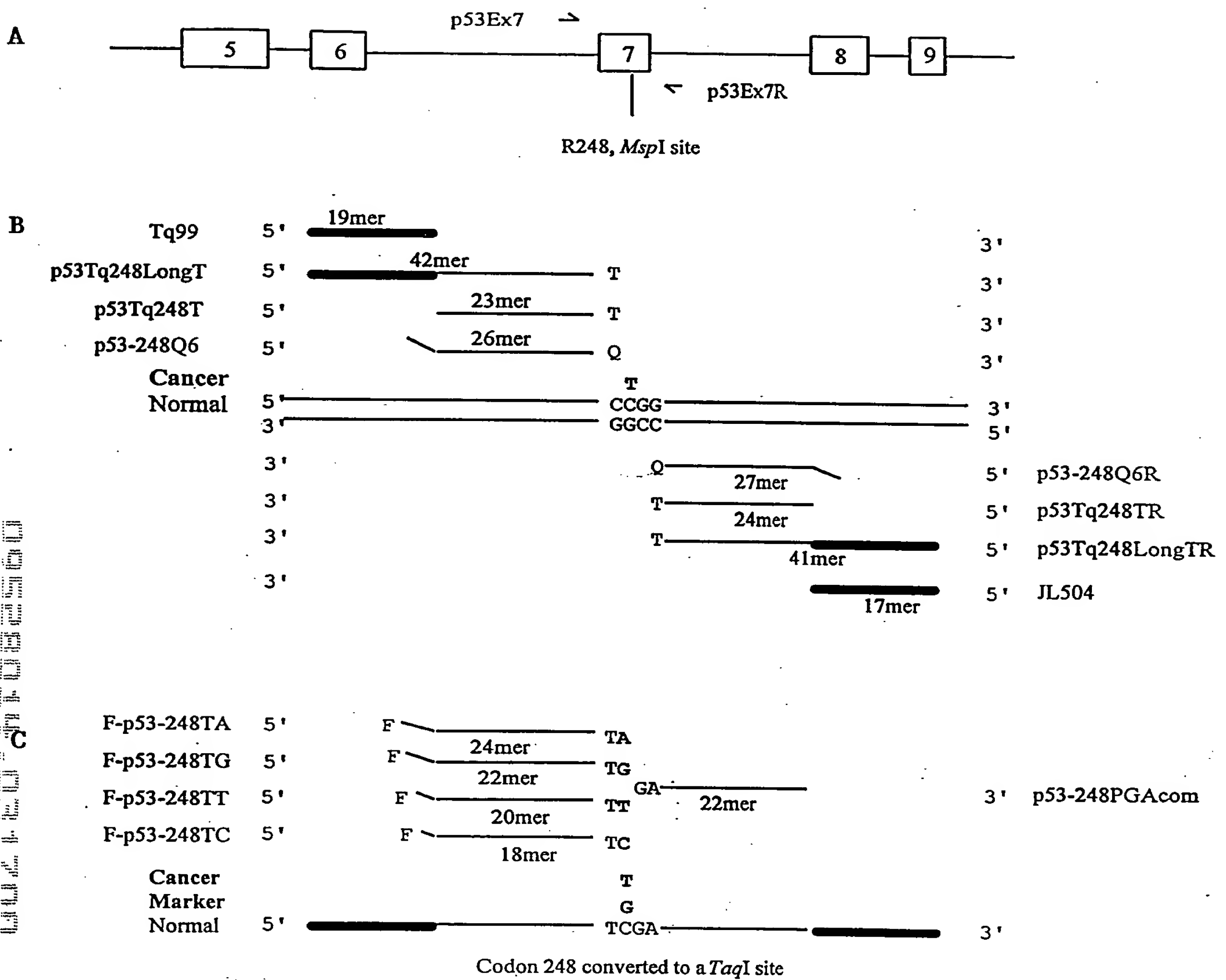


FIGURE 10

### Primers

Ztop CTT GGA OGA GTT CAT ACG C  
 ↓  
 p53zip248short CTT GGA OGA GTT CAT ACG CGT TOC TGC ATG GGC GGC ATG A  
 p53-248short GT TOC TGC ATG GGC GGC A→*pol*  
 || ||| ||| ||| ||| ||| ||| |  
 p53 exon 7 3' ...CA AGG AGC-TAC CCG CCG TAC TTG GGC TOC GGG TAG GAG TGG TAG TAG TGT... 5' (-)  
 PCR product 5' ...GT TOC TGC ATG GGC GGC ATG AAC GGG AGG CCC ATC CTC ACC ATC ATC ACA... 3' (+)  
 (MK not shown) || ||| ||| ||| ||| ||| ||| |||  
 p53-248shortR *pok*-GG TAG GAG TGG TAG TAG TG  
 p53zip248shortR C GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC C  
 Zbot C ACC GCT GGG TCA AAC C

# B

## Primers

Ztop  
 p53zip248T  
 p53Taq248T  
 p53Taq248Q<sub>6</sub>  
 50-bp synthetic  
 duplex DNA, or  
 PCR product  
 p53Taq248Q<sub>6</sub>R  
 p53Taq248TR  
 p53zip248TR  
 Zbot

CTT GGA OGA GTT CAT ACG C  
 CTT GGA OGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG AAT  
 GT TCC TGC ATG GGC GGC ATG AAT  
 TTCT TCC TGC ATG GGC GGC ATG AAQ<sub>6</sub>→*pol*  
 | ||| ||| ||| ||| ||| ||| ||| :  
 3' CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)  
 5' GT TCC TGC ATG GGC GGC ATG AAC GGG AGG CCG ATC CTC ACC ATC ATC ACA 3' (+)  
 : ||| ||| ||| ||| ||| ||| ||| |  
*pol*-Q<sub>6</sub>TCC GGG TAG GAG TGG TAG TAG TCCT  
 T TCC GGG TAG GAG TGG TAG TAG TG  
 T TCC GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC G  
 C ACC GCT GGG TCA AAC G



### LDR Primers

p53LDR248FTCL F-AAAAAAAA GC ATG GGC GGC ATG AAT C

p53LDR248FCA F-AAAAAA GC ATG GGC GGC ATG AAC A

p53LDR248FCG F-AAAA GC ATG GGC GGC ATG AAC G

p53LDR248FCT F-AA GC ATG GGC GGC ATG AAC T

p53LDR248FCC F- GC ATG GGC GGC ATG AAC C 7-ligase

**p53LDR248PGG**

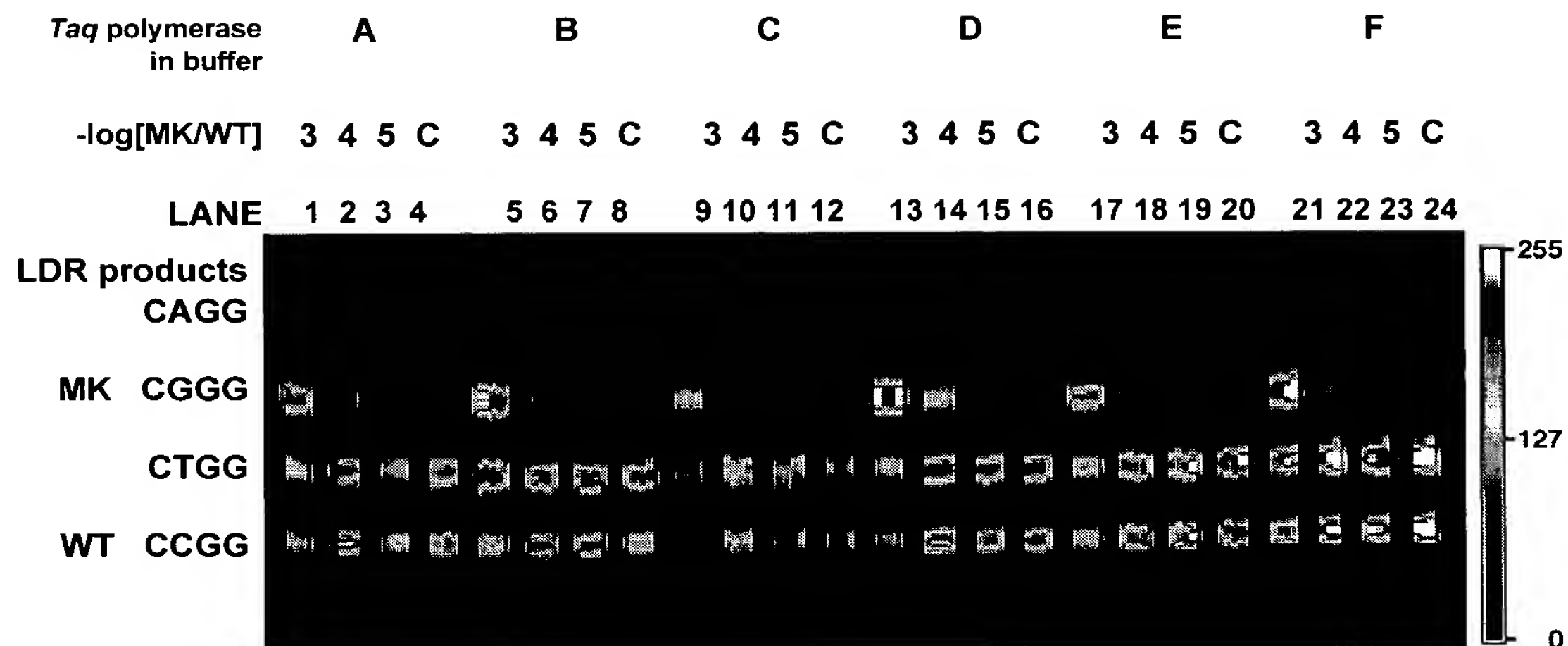
conversion products

3' (-strand)      || ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||      5'

... GTA TGC GCA AGG ACG TAC CCG CCG TAC TTG NGG TCC GGG TAG GAG TGG TAG TAG TGA ACC...

FIGURE 11

**A**



**B**



FIGURE 12

